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(54) Title: SEGMENTED CHELATING POLYMERS AS IMAGING AND THERAPEUTIC AGENTS

(57) Abstract

A segmented polymer suitable for use in diagnostic imaging or as a cell killing agent comprising a chelating agent residue linked via an amide linkage to a poly(alkylene oxide) moiety, said polymer having a molecular weight of at least 4,500.

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SEGMENTED CHELATING POLYMERS AS IMAGING AND THERAPEUTIC AGENTS

This invention relates to segmented chelating polymers which are useful as imaging agents and as cytotoxic agents and to their compositions and methods of use.

Magnetic resonance (MR) is a widely used technique for generating spatial images of multi-cellular organisms or bodies for clinical diagnosis. A review of this technique and its clinical applications is provided by D.P Swanson et al, in Pharmaceuticals in Medical Imaging, 1990, Macmillan Publishing Company, pages 645-681.

Magnetic resonance images are derived instrumentally as a composite of the effects of a number of parameters which are analyzed and combined by computer. Instrument parameter controls, such as radio frequency (Rf), pulsation and timing can be utilized to enhance or attenuate the signals of any of the image-producing parameters to thereby improve image quality and provide better anatomical and functional information. In many cases, magnetic resonance imaging has proven to be a valuable diagnostic tool, inasmuch as normal and diseased tissue, by virtue of their possessing different responses to parameter values, can be differentiated in the image.

In magnetic resonance imaging of the body of a mammal such as a human, an in vivo image of an organ or tissue is obtained by placing at least the portion of the body to be imaged in a strong external magnetic field, pulsing with radiofrequency energy, and observing the effect of the pulses on the magnetic properties of the protons contained in and surrounding the organ or tissue. This is especially useful in imaging the

circulatory vasculation of the body (i.e. the blood pool). A number of magnetic parameters can be measured. In particular, the proton relaxation times, T_1 and T_2, are of primary importance. T_1, also called the spin-lattice or longitudinal relaxation time, and T_2, also called the spin-spin or transverse relaxation time, are functions of the chemical and physical environment of the organ or tissue water and are measured using well-established Rf pulsing techniques. This information is analyzed as a function of spatial location and transformed by computer into an image.

Often the MR image produced lacks appropriate contrast, e.g., between normal and diseased tissue, reducing diagnostic effectiveness. However, the contrast in the image generated may be enhanced by introducing into the zone being images an agent (a "contrast agent") which affects the spin reequilibration characteristics of nuclei (the "imaging nuclei" which generally are protons and more especially are water protons) which are responsible for the resonance signals from which the images are generated. The enhanced contrast thus obtained enables particular organs or tissues to be visualised more clearly by increasing or decreasing the signal level of the particular organ or tissue relative to its surroundings. In other words, a contrast agent, if taken up preferentially by a certain portion of an organ or a certain type of tissue, e.g., diseased tissue, can provide a change in contrast or enhancement in the resultant images of that tissue.

Inasmuch as magnetic resonance images are strongly affected by variations in T_1 and T_2, it is desirable to have a contrast agent which affects either or both of these parameters. The majority of materials now being proposed as MR imaging contrast agents achieve a contrast effect because they contain paramagnetic materials, which generally act to decrease T_1 and T_2, or superparamagnetic materials, which generally act to

decrease T_2. At low concentrations, paramagnetic materials effect T_1 more than T_2. The use of such materials as MR contrast agents has been widely advocated and broad ranges of suitable materials have been suggested in the literature.

Thus, for example Lauterbur and others have suggested the use of manganese salts and other paramagnetic inorganic salts and complexes (see Lauterbur et al. in "Frontiers of Biological Energetics", volume 1, pages 752-759, Academic Press (1978), Lauterbur in Phil. Trans. R. Soc. Lond. B289: 483-487 (1980) and Doyle et al. in J. Comput. Assist. Tomogr. 5(2): 295-296 (1981)), Runge et al. have suggested the use of particulate gadolinium oxalate (see for example US-A-4615879 and Radiology 147(3): 789-791 (1983)), Schering AG have suggested the use of paramagnetic metal chelates, for example of aminopolycarboxylic acids such as nitrilotriacetic acid (NTA), N,N,N'N'-ehtylenediaminetetraacetic acid (EDTA), N-hydroxyethyl-N,N'N'-ethylenediaminetriacetic acid (HEDTA), N,N,N',N",N"-diethylenetriaminepentaacetic acid (DTPA), and 1,4,7,10-tetraazacyclododecanetetraacetic acid (DOTA) (see for example EP-A-71564, EP-A-130934, DE-A-3401052 and US-A-4639365), and Nycomed Imaging AS and Nycomed Salutar Inc. have suggested the use of paramagnetic metal chelates of iminodiacetic acids and other aminopolycarboxylic acids such as DTPA-BMA and DPDP (see EP-A-165728, WO-A-86/02841, EP-A-299795, EP-A-290047 and WO-A-90/08138). Besides paramagnetic metals, paramagnetic stable free radicals have also been suggested for use as positive MR imaging contrast agents (see for example EP-A-133674).

Other paramagnetic MR contrast agents are suggested or reviewed in, for example, EP-A-136812, EP-A-185899, EP-A-186947, EP-A-292689, EP-A-230893, EP-A-232751, EP-A-255471, WO-A-85/05554, WO-A-86/01112, WO-A-87/01594, WO-A-87/02893, US-A-4639365, US-A-4687659, US-A-4687658,

AJR 141: 1209-1215 (1983), Sem. Nucl. Med. 13: 364 (1983), Radiology 147: 781 (1983), J. Nucl. Med. 25: 506 (1984) and WO89/00557.

Superparamagnetic MR contrast agents were disclosed by Schröder and Salford in WO-A-85/02772, by Nycomed AS in WO-A-85/04330, by Widder in US-A-4675173, by Schering AG in DE-A-3443252 and by Advanced Magnetics Inc. in WO-A-88/00060.

A major drawback with the use of contrast agents for magnetic resonance imaging is that at effective dosages many paramagnetic materials exert toxic effects on biological systems making them inappropriate for *in vivo* use. For example, the free solubilized form of Gd^{3+} salts are quite toxic. To make the gadolinium ion more suitable for in vivo use, researchers have chelated it using diethylenetriaminepentaacetic acid (DTPA). This agent, GdDTPA, has been successful in enhancing magnetic resonance images of human brain and renal tumors.

Despite its satisfactory relaxivity and safety, this formulation has several disadvantages. For example, due to its low molecular weight, Gd-DTPA is cleared very rapidly from the blood stream and tissue lesions (tumors). This limits the imaging window, the number of optimally enhanced images that can be obtained after each injection, and increases the agent's required dose and its toxic effects. In addition, the biodistribution of Gd-DTPA is suboptimal for imaging body tumors and infections due to its low molecular weight.

Several approaches have been taken in attempts to overcome these disadvantages. For example, Gd^{3+} and Gd^{3+} chelates have been chemically conjugated to proteins such as albumin, polylysines and immunoglobulins. Drawbacks of conjugating for example DTPA to protein carriers include inappropriate biodistribution and toxicity. In addition, available proteins are not subject to wide synthetic variation. Additionally, thermal sterilization of protein conjugates tends to be

problematic, especially in the case of albumin conjugates.

Furthermore proteins are metabolized by the body, providing an imaging agent whose molecular weight changes uncontrollably. This causes blood pool half-life tissue specificity of the imaging agent to change continuously. Proteins are immunogenic substances, which have several known drawbacks for therapeutic or diagnostic use. Solutions to these recognized problems are clearly of importance generally in the imaging field.

There is a need to provide new classes of magnetic resonance contrast enhancing agents, which are tissue specific, and which remain in the blood pool for long periods of time.

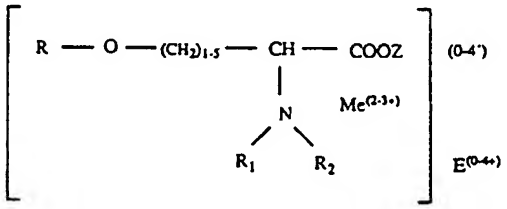
The importance of drug targeting has been recognized in recent years, especially for anticancer drugs, inasmuch as the toxicity of these drugs to normal cells is often dosage limiting. Drug targeting has been employed in this area, using antibodies to tumor associated antigens or using other proteins and saccharides to avoid the random destruction of healthy tissue. Antibodies and portions or fragments thereof have been most widely used due to their specificity. The current approaches, however, are expensive and pose immunogenicity problems. Furthermore, not all cancers are susceptible to drug therapy.

Certain tumors are especially susceptible to treatment by radiation, such as those of hematopoietic origin, for example, leukemias and lymphomas; others are less susceptible to such treatment such as adenocarcinomas of the head and neck, or breast, ovarian, cervical or rectal adenocarcinomas. However, certain of these cancers cannot be practically treated by externally derived beam radiation because of the location of the tumor and the effect of such radiation on surrounding healthy tissue. Hence, it is advantageous

to deliver a source of radiation such as a radioactive isotope to the tumor, whilst minimizing damage to surrounding healthy tissues which in conventional treatment may lie in the path of the beam. It would be advantageous to deliver the appropriate radiation dosage directly to the tumor with significantly less harm to the surrounding tissue thereby increasing the therapeutic ratio (the ratio of damage to the tumor divided by damage to the most sensitive healthy tissue).

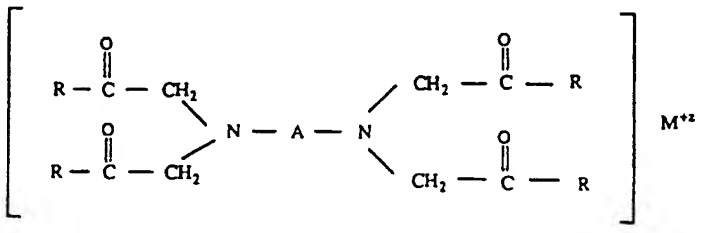
In summary, it would be desirable to prepare a material that would overcome the following disadvantages; 1) toxicity, 2) short blood pool residence time, 3) lack of specificity for targeted tissues, 4) immunogenicity, and 5) uncontrolled metabolism of the polymer. Several attempts have been made to provide such a polymer.

Felder et al in U.S. Patent No. 4,916,246 disclose low molecular weight paramagnetic chelates useful for NMR imaging having the formula:

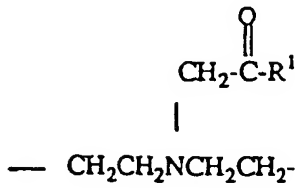


wherein Me is Fe, Mn, Dy or Gd, Z is H or a negative charge, R_1 and R_2 are substituted alkyl and R can be poly(oxy-alkyl) with 1 to 50 oxygen atoms and from 3 to 150 carbon atoms.

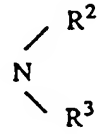
U.S. Patent No. 5,137,711 describes low molecular weight complexes of paramagnetic ions with derivatives of DTPA or EDTA represented by the formula:



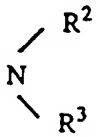
wherein A is selected from the group consisting of $-CH_2CH_2-$ and:



and M^{z+} is a paramagnetic ion of an element with an atomic number of 21-29, 42-44 or 58-70, and a valence, Z, of +2 or +3; the R^1 and R groups may be the same or different and are selected from the group consisting of $-O^-$ and



wherein R^2 is $(CH_2CH_2O)_n-R^4$, n is 1-10 and R^4 is H, alkyl having 1 to 8 carbon atoms (i.e. C_{1-8}) or aryl, unsubstituted or substituted with hydroxy, and R^3 is H, R^2, C_{1-8}-alkyl, hydroxy, C_{1-8}-alkoxy, C_{1-10}-cycloalkyl or an aryl group which is optionally substituted with hydroxy, carboxy, halide, C_{1-8}-alkoxy or C_{1-8}-alkyl, wherein 2 of the R^1 groups are $-O^-$ and the remainder of the R^1 groups are groups



International Patent Publication No. WO 91-18630 discloses substances for treating or diagnosing tumors, having at least one aliphatic amino group, or at least one phenolic hydroxyl and/or amino group and at least

one aliphatic amino group, all substituted with polyethylene glycol chains, whose degree of polymerization, n, is 5 to 250. The terminal hydroxyl group of the chain is substituted by C_{1-12} alkyl ester.

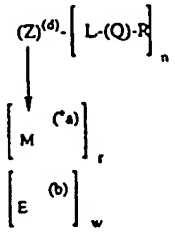
Herz, et al. in U.S. Patent No. 3,859,337 disclose low molecular weight ethylenediamine tetraacetic acid anhydride derivatives useful as chelating agents.

It has now been found that certain metallizable segmented polymers when associated with metal ions provide higher molecular weight chelates of surprising utility as blood pool contrast agents for diagnostic imaging and/or as cytotoxic agents.

Thus viewed from one aspect, the present invention provides a segmented polymer having a molecular weight of at least about 4,500 comprising at least one poly(alkylene oxidylene) moiety linked via an amide linkage to a chelating agent residue.

Especially preferred segmented polymers according to the invention are those wherein said chelating agent residue in metallated, preferably with a paramagnetic metal species or with a radionuclide species.

Particularly preferred segmented polymers according to the invention are those of Formula I:



(wherein:

Z is a chelating agent residue;

Q is a divalent poly(alkylene oxidylene) moiety having a carbon terminus at R and at L;

L represents an amide linkage;

$E^{(b)}$ is one or more counterions each having a charge of b;

b is an integer from 1, 2 and 3;

n is an integer from 1, 2, 3 and 4;
w is zero or an integer from 1, 2 3,4 and 5;
$M^{(+a)}$ is a cation, having a charge of +a;
a is an integer from 1, 2, 3 and 4;
r is 0 or an integer from 1, 2 and 3, with the proviso that that when r is 2 or 3, each $M^{(+a)}$ can be the same or different;
d is the total charge on the chelating agent residue and is an integer from 0 to 10;
$d + \Sigma(b.w) + \Sigma(a.r) = 0$; and
R is a capping moiety selected from the group consisting of hydrogen, hydroxyl, C_{1-4}-alkyl, C_{6-24}-aryl, C_{2-5}-alkanoyloxyl and C_{1-4}-alkoxy, or R is an immunoreactive group or cytotoxic drug linked to group Q by a chemical bond or a linking group).

The segmented polymers of the invention have a variety of end uses. In particular they may be used as diagnostic agents, eg. image contrast enhancing agents in diagnostic imaging techniques such as MRI, X-ray and scintigraphy, as therapeutic agents, for example in radiotherapy or drug delivery, or as targetting agents, for example in cytotoxic or imaging procedures or in fluorescence imaging. The chelating residues may be metallated with metal species useful diagnostically or therapeutically, such as paramagnetic or radioactive metal ions. The targetting delivery system effected by the segmented polymers of the invention is of course also especially useful for delivery of metal species useful in diagnostic imaging of body organs or tissues or as cytotoxic agents.

Accordingly, viewed from a further aspect the present invention provides a pharmaceutical composition comprising a segmented polymer according to the invention together with at least one physiologically acceptable carrier or excipient.

Moreover, the segmented polymers according to the invention as well as being particularly useful as image

enhancing agents, are particularly useful in chelation therapy for metal poisoning, especially heavy metal poisoning and in detecting such ions, and have the added advantage of being able to remain in the vascular system for remarkably long periods of time. Certain segmented polymers have tissue specificity, e.g. tumour or liver specificity. Indeed, the molecular weight of the segmented polymers may be tailored to produce an agent of a desired overall molecular weight, size, blood pool residence time and tissue specificity. Furthermore, the segmented polymers according to the invention may be useful in detection of flows and leaks in materials that contain liquids, and in apparatus used with liquids for heating, cooling, lubrication and the like.

The segmented polymers of the invention may be prepared by a particularly elegant and simple reaction of a poly(alkylene oxide) with a chelating agent or precursor thereof.

Thus viewed from a different aspect the invention provides a process for the preparation of a segmented polymer, said process comprising reacting a poly(alkylene oxide) with a chelating agent or precursor thereof.

As used herein "segmented" polymer means a polymer having one component or "segment" comprising a chelating agent residue Z and from one to four regions or "segments" each comprising a capped or uncapped poly(alkylene oxidylene) moiety.

As used herein the term "body" refers to a bounded aggregate of matter, preferably the entire material substance or organism, especially an animal, most preferably an animal or human subject.

As used herein "cytotoxic agent" refers to any agent able to kill cells, including a radionuclide, whose emissions lead to cell death including a chelate of a radionuclide that emits cytotoxic radiation, chemotherapeutic agents such as cytotoxic drugs and

cytotoxic antibiotics, toxins or any agent which initiates or which leads to cell death. In a preferred embodiment, the cytotoxic agent is a metal ion associated with the chelating agent residue.

The segmented polymer according to the invention can have an imaging ion associated therewith. By "imaging ion", it is meant any metal ion useful for enhancing contrast for example during x-ray, radioscintigraphic, fluorescence or magnetic resonance imaging. The imaging agent may conveniently be ionically associated with the chelating agent residue. Different types of metal ions may be employed in the same polymer so as to be useful for different types of imaging simultaneously, (for example, the ions of different elements, or different isotopes of the same element) or the same ion (e.g. ions of the same element or isotopes of the same elements) may be imaged by different methods.

As used herein aryl refers to monocyclyl to hexacyclyl aryl, having from 6 to 24 carboxy atoms and may be additionally substituted with one or more C_{1-4}-alkoxy, and/or C_{1-4}-alkyl groups. Such aryl groups, when used in the polymer according to the invention are useful for fluorescence detection.

In formula I above, Q represents a poly(alkylene oxidylene) moiety having a carbon terminus at L and a carbon terminus at R and containing m alkylene oxide units. Exemplary poly(alkylene oxidylene) moieties comprise, for example, poly(ethylene oxides), and/or poly(propylene oxides) and/or poly(butylene oxides) and the like. Preferred poly(alkylene oxidylene) moieties include poly(ethylene oxidylene) moieties (PEO), poly(propylene oxidylene) (PPO) and random and block copolymers of PEO and PPO. PEO containing polymers are particularly preferred when it is desirable for the segmented polymer to be soluble in water. It is also contemplated that the poly(alkylene oxidylene) moiety

can comprise glycerol poly(alkylene oxide) triethers, polyglycidols, linear, block and graft copolymers of alkylene oxides with compatible comonomers such as poly(ethyleneimine-co-ethylene oxide), or poly(oxazoline-co-alkyleneoxide) and grafted block copolymers such as poly(methyl vinyl ether-co-ethylene oxide.

For magnetic resonance imaging applications, segmented polymers according to the invention preferably have an average molecular weight greater than about 4,500, more preferably about 4,500 to about 40,000. These polymers can be derived from poly(alkylene oxidylene) moieties which are commercially available in the corresponding alcohol form as a diol, or as a monoalkyl ether alcohol, or monoalkyl ether monoamine form, or alternatively they can be prepared by techniques well known to those skilled in the art. The number of alkylene oxide subunits in Q depends upon 1) n, the number of L-Q-R segments extending from Z; 2) upon the "molecular" weight of each alkylene oxide subunit moiety; 3) the desired molecular weight of the polymer; 4) the molecular weight of R and Z, as well as $M^{(a+)}$. If n is greater than 1, each poly(alkylene oxide) moiety may be the same or different. Expressed mathematically this is: n x [(MW of L & R) + (MW of alkylene oxide subunit x m)] + (r x atomic weight of chelated metal(s)) + MW of chelating residue + molecular weight of Z = MW of the polymer. Thus m is a function of n for a given molecular weight of the polymer. A particularly preferred class of poly(alkylene oxide) moieties derived from poly(ethylene oxides) can be represented by the structure:

$$-(CH_2CH_2O)_mCH_2CH_2-$$

If four PEO chains are present (n=4) then the upper limit of the most preferred molecular weight of 40,000

will be obtained with m= about 220. If a molecular weight of 5,000 is desired in the same type of polymer (n=4) then m will be closer to 30.

These poly(alkylene oxide) moieties and their reactive derivatives, useful in preparing the segmented polymer of the invention, are known in the art. For example, bis(methyl amino) polyethylene glycol and its use as an intermediate is known in the art, for example; Mutter, Tetrahedron Letters, 31, 2839-2842 (1978) describes a procedure to convert the terminal hydroxyl groups of poly(ethylene oxide) to reactive primary amino groups as well as the preparation of a number of reagents bound to poly(ethylene oxide) amines; Harris et al., J. Polymer. Science, 22, 341-352 (1984) describe various PAG derivatives including for example, amino poly(ethylene oxide). Other poly(alkylene oxide) derivatives are prepared by known chemistries, examples of which are described previously.

As is well known, a chelating molecule is a compound containing electron donor atoms that can combine by coordinate bonding with a cation to form a coordination complex or chelate. This class of compounds is described in the Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 5, 339-368. By analogy, in the segmented polymer according to the invention the chelating agent residue is typically a chelating moiety which is a radical or a multivalent radical, rather than a molecule or a compound in itself. The polymer incorporating the chelating agent residue could thus be described as a chelating polymer.

In structure 1 above, Z represents a chelating agent residue. The chelating agent residue can be derived and/or selected from moieties which contain electron donor atoms. These moieties can be selected from, for example; polyphosphates, such as sodium tripolyphosphate and hexametaphosphoric acid;

linear, branched or cyclic aminocarboxylic acids,

such as ethylenediaminetetraacetic acid, N(2-hydroxyethyl)ethylenediaminetriacetic acid, nitrilotriacetic acid, N,N-di(2-hydroxyethyl)glycine, ethylenebis(hydroxyphenylglycine) and diethylenetriamine pentaacetic acid and the N-carboxymethylated macrocyclic polyazacycloalkanes such as DOTA and DO3A and the phosphonomethylated analogues;

1,3-diketones, such as acetylacetone, trifluoroacetylacetone, and thienoyltrifluoroacetone;

and hydroxycarboxylic acids, such as tartaric acid, mucic acid, citric acid, gluconic acid, and 5-sulfosalicylic acid;

polyamines, such as ethylenediamine, diethylenetriamine, triethylenetetramine, and triaminotriethylamine;

aminoalcohols, such as triethanolamine and N-(2-hydroxyethyl) ethylenediamine;

aromatic heterocyclic bases, such as 2,2'-dipyridyl, 2,2'-diimidazole, dipicoline amine and 1,10-phenanthroline;

phenols, such as salicylaldehyde, disulfopyrocatechol, and chromotropic acid;

aminophenols, such as 8-hydroxyquinoline and oxinesulfonic acid;

oximes, such as dimethylglyoxime and salicylaldoxime;

peptides containing proximal chelating functionality such as polycysteine, polyhistidine, polyaspartic acid, polyglutamic acid, or combinations of such amino acids, each polyamino acid containing from 2 to about 20 amino acids in the polymer;

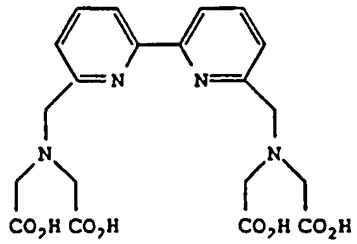
Schiff bases, such as disalicylaldehyde 1,2-propylenediimine;

tetrapyrroles, such as tetraphenylporphin and phthalocyanine;

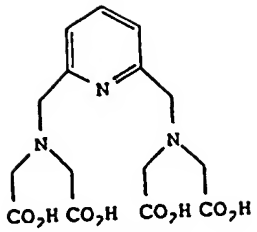
sulfur compounds, such as toluenedithiol, meso-2,3-dimercaptosuccinic acid, dimercaptopropanol, thioglycolic acid, potassium ethyl xanthate, sodium diethyldithiocarbamate, dithizone, diethyl

dithiophosphoric acid and thiourea;
synthetic macrocylic compounds, such as dibenzo[18]crown-6, $(CH_3)_6$-[14]-4,11-diene-N_4, and (2.2.2)-cryptate; and
phosphonic acids, such as nitrilotrimethylenephosphonic acid, ethylenediaminetetra(methylenephosphonic acid), and hydroxyethylidenediphosphonic acid, or combinations of two or more of the above agents.

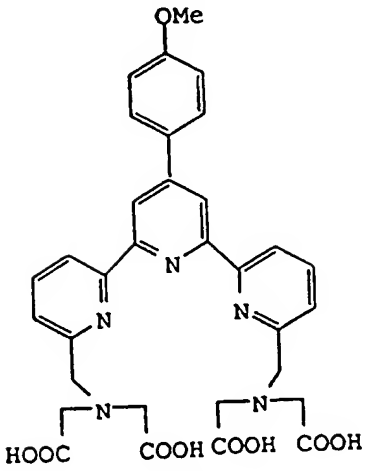
Preferred chelating agent residues contain one or more carboxylic acid or carboxylate groups and include elements present in: ethylenediamine-N, N, N',N'-tetraacetic acid (EDTA); N, N, N', N", N"-diethylenetriaminepentaacetic acid (DTPA); 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA); 1,4,7-10-tetraazacyclododecane-N,N',N"-triacetic acid (DO3A); 1-oxa-4,7,10-triazacyclododecane-N,N',N"-triacetic acid (OTTA);
trans(1,2)cyclohexanodiethylenetriamine pentaacetic acid (CDTPA);



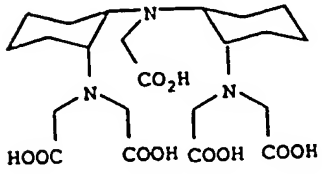
(B4A);



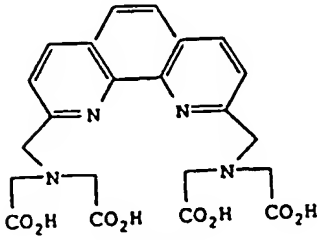
(P4A);



(TMT);



(DCDTPA) ; and



(PheMT).

Such chelating agent compounds, including their preparation and chemical manipulation are well known in the art. For example, the acid and anhydride forms of EDTA and DTPA are commercially available. Methods for preparing B4A, P4A and TMT are described in U.S. Patent 4,859,777. Other suitable chelating groups are known in the art, and are described for example in PCT/US91/08253.

If Z is a chelating agent residue made of multiple chelating moieties or subunits, each of the subunits can be linked together by a linking group. Thus, more than one chelating moiety can be used to make up the chelating agent residue. If more than one chelating moiety is present in the chelating agent residue, these may be the same or different. Chelating moieties can be linked together using known chemistries and materials. Thus the chelating agent residue can be one moiety or a "core" of chelating moieties. For example, a core of DTPA residues may be prepared by reacting DTPA dianhydride with a diamine, such as ethylene diamine, to form a "core" of DTPA chelators. The anhydrides react with the amine to form amide bonds. Two DTPA moieties linked by one ethylene diamine and three DTPA moieties linked by 2 ethylene diamines are examples of preferred "cores" containing multiple DTPA residues, others are contemplated. Other chelating agent residues, made up of multiple chelating moieties are well known in the art and are prepared by known chemistries.

The segmented polymer according to the invention can be capped at the termini with groups represented by R in formula I linked to the termini of the poly(alkylene oxide) alkylene groups by a chemical bond or a linking group. These can be independently selected from hydrogen, hydroxy, C_{1-4}-alkyl, aryl as hereinbefore defined, carboxy, C_{2-3}-alkanoyloxy, and C_{1-4}-alkoxy. In preferred embodiments, the polymer can be capped by reagents that produce acyl derivatives, for example, by

monoanhydrides, e.g., acetic anhydride or succinic anhydride and iodoacetic acid anhydride which forms an iodomethyl carbonyloxy intermediate that can be further reacted with an R precursor such as a protein or cytotoxic agent.

R in formula I can also be a cytotoxic drug, for example, a cytotoxic drug useful in the treatment of cancer. The cytotoxic drug will be selected with reference to factors such as, for example, the type of cancer tumor and the efficacy of a certain chemotherapy agent for treating the cancer tumor involved. The chemotherapy agent may be selected from alkylating agents, antimetabolites, natural products useful as cytotoxic drugs, hormones and antagonists and other types of cytotoxic compounds.

Examples of alkylating agents include the nitrogen mustards (i.e. the 2-chloroethylamines) such as, for example, chloromethine, chlorambucil, melphalan, uramustine, mannomustine, extramustine phosphate, mechlor-thaminoxide, cyclophosphamide, ifosamide and trifosfamide; alkylating agents having a substituted aziridine group such as, for example, tretamine, thiotepa, triaziquone and mitomycin; alkylating agents of the alkyl sulfonate type; such as, for example, busulfan, hepsulfam, and piposulfan; alkylating N-alkyl-N-nitrosourea derivatives such as, for example, carmustine, lomustine, semustine or streptozotocine; alkylating agents of the mitobronitole, dacarbazine and procarbazine type; and platinum complexes such as, for example, cisplatin and carboplatin and others.

Examples of antimetabolites include folic acid derivatives such as, for example, methotrexate, aminopterin and 3'-dichloromethotrexate; pyrimidine derivatives such as, for example, 5-fluorouracil, floxuridine, tegafur, cytarabine, idoxuridine, and flucytosine; purine derivatives such as, for example, mercaptopurine, thioguanine, azathioprine, tiamiprine,

vidarabine, pentostatin and puromycin and others.

Examples of natural products useful as cytotoxic agents include for example vinca alkaloids, such as vinblastine and vincristine; epipodophylotoxins such as, for example, etoposide, and teniposide; antibiotics such as, for example, adrimycin, daunomycin, dactinomycin, daunorubicin, doxorubicin, mithramycin, bleomycin and mitomycin; enzymes such as, for example, L-asparaginase; biological response modifiers such as, for example, alpha-interferon; camptothecin; taxol; and retinoids such as retinoic acid and the like.

Examples of hormones and antagonists include adrenocorticoids, such as, for example, prednisone; progestins, such as, for example, hydroxyprogesterone acetate, medroxyprogesterone acetate and megestrol acetate; estrogens such as, for example, diethylstilbestrol and ethinyl estradiol; antiestrogens such as for example, tamoxifen; androgens such as, for example, testosterone propionate and fluoxymestrone; antiandrogens such as, for example, flutamide; and gonadotropin-releasing hormone analogs such as, for example, leuprolide.

Examples of miscellaneous agents include anthracenediones such as for example, mitoxantrone; substituted ureas such as, for example, hydroxyureas; and adrenocortical suppressants such as, for example, mitotane and aminoglutethimide.

In some embodiments, R is an immunoreactive group covalently bonded to Q through a linking group. As used herein, the term "immunoreactive group" is meant to include an organic compound which is capable of being covalentiy bound to the polymer and which is found in a living organism or is useful in the diagnosis, treatment or genetic engineering of cellular material or living organisms, and which has a capacity for interaction with another component which may be found in biological fluids or which may be associated with cells to be

treated such as tumor cells.

Depending upon the intended use, the immunoreactive group can be selected from a wide variety of naturally occurring or synthetically prepared materials, including, but not limited to enzymes, amino acids, peptides, polypeptides, proteins, lipoproteins, glycoproteins, hormones, drugs (for example digoxin, phenytoin, phenobarbitol, thyrozine, triiodothyronine, gentamicin, carbamazepine, and theophylline), steroids, vitamins, polysaccharides, viruses, protozoa, fungi, parasites, rickettsia, molds, and components thereof, blood components, tissue and organ components, pharmaceuticals, haptens, lectins, toxins, nucleic acids (including oligonucleotides), antibodies, antigenic materials (including proteins and carbohydrates), avidin and derivatives thereof, biotin and derivatives thereof, histones and others known to one skilled in the art.

Preferred immunoreactive groups (sometimes referred to in the art as ligands) are those which have a receptor molecule specific to the ligand of interest. Thus, a specific binding reaction involving ligand, R, and receptor to form a ligand-receptor complex can be used for targeting, and treatment, and for imaging a target site. Examples of such ligand-receptor complexes include, but are not limited to antibody-antigen, avidin-biotin, histone-DNA, repressor (inducer), promoter of operons and sugar-lectin complexes. Additionally, complementary nucleic acids, both natural and antisense are also considered specific binding materials as the term is used herein. Either component of the above listed pairs can be useful as a ligand if the other component is found or attached to the target site.

Useful immunoreactive groups include (1) any substance which, when presented to an immunocompetent host, will result in the production of a specific antibody capable of binding with that substance, or (2)

the antibody so produced, which participates in an antigen-antibody reaction. Thus, the immunoreactive group can be an antigenic material, an antibody, or an anti-antibody. Both monoclonal and polyclonal antibodies are useful. The antibodies can be whole molecules or various fragments thereof, as long as they contain at least one reactive site for reaction with the reactive groups on the complexing agent or with linking groups as described herein.

In certain embodiments, the immunoreactive group can be an enzyme which has a reactive group for attachment to the complexing agent. Representative enzymes include, but are not limited to, aspartate aminotransaminase, alanine aminotransaminase, lactate dehydrogenase, creatine phosphokinase, gamma glutamyl transferase, alkaline acid phosphatase, prostatic acid phosphatase, tissue plasminogen activator, horseradish peroxidase and various esterases.

If desired, the immunoreactive group can be modified or chemically altered to provide reactive groups for linking the immunoreactive group to the polymer. These methods are well known in the art. Such techniques include the art recognized use of linking moieties and chemical modification such as described in WO-A-89/02931 and WO-A-89/2932, which are directed to modification of oligonucleotides, and the disclosure of U.S. Patent 4,719,182. Where the reactive group for linking the immunoreactive group to the polymer is attached to a protein, antibody and the like, it is called a "protein reactive group"

Two highly preferred uses for the compositions of this invention where R is an immunoreactive group are for the diagnostic imaging of, tumors and the radiological treatment of tumors. Preferred immunoreactive groups therefore include antibodies, or immunoreactive fragments thereof, to tumor-associated antigens. Specific examples include B72.3

antibodies (described in U.S. Patent Nos. 4,522,918 and 4,612,282) which recognize colorectal tumors, 9.2.27 antimelanoma antibodies, D612 antibodies which recognize colorectal tumors, UJ13A antibodies which recognize small cell lung carcinomas, NRLU-10 antibodies which recognize small cell lung carcinomas and coalorectal tumors (Pan-carcinoma), 7E11C5 antibodies which recognize prostate tumors, CC49 antibodies which recognize colorectal tumors, TNT antibodies which recognize necrotic tissue, PR1A3 antibodies, which recognize colon carcinoma, ING-1 antibodies, which are described in International Patent Publication WO-A-90/02569, B174 antibodies which recognize squamous cell carcinomas, B43 antibodies which are reactive with certain lymphomas and leukemias and others which may be of particular interest.

Such antibodies and other useful immunological groups described above are large, complex protein molecules having multiple sites for appendage of the segmented polymer complexing. Consequently, the immunoreactive group can have appended to it additional complexing agents each via one of the protein reactive groups. Thus, the term immunoreactive group is intended to include immunological groups having at least one segmented polymer molecule bonded thereto through at least one protein reactive group.

Additionally, the immunoreactive group can be an antibody or fragment thereof containing a carbohydrate region which can be attached to the polymer through the carbohydrate region such as described in U.S. Patent 4,937,183. Useful methods for attaching an antibody are also described in U.S. Patent Nos. 4,671,958; 4,699,784; 4,741,900; and 4,867,973.

Additionally, immunoreactive groups that interact with intracellular components are also specifically contemplated; for example, actin, myosin, histone, DNA, DNAase and the like. These molecules, while useful in

all contemplated applications, are preferred for targeting solid tumors and necrotic cells. Specifically contemplated in this embodiment is the use of a histone linked to the polymer of the invention for imaging solid tumors. Histones have low immunogenicity, bind tightly to nucleic acids, and have difficulty passing through cell and nuclear membranes in intact living cells. However, in necrotic tissue, the cell and nuclear membranes no longer function and are often perforate, allowing entry of foreign materials, including histones. This diffusion into necrotic tissue can be used to accumulate an imaging and/or cytotoxic agent of this invention in the vicinity of cancer cells, especially in solid tumors. Necrosis can occur naturally due to a limited blood supply, or can be induced by cytotoxic agents or irradiation. Thus, this method is useful in imaging and/or treatment of tumors. As a further example, DNAase when attached to the polymer of the invention can be used to target for detection and destruction, free DNA.

Free DNA accumulates in certain diseases, such as systemic lupns where it ultimately causes kidney failure. Thus this polymer can be used to detect DNA accumulation in kidney glomeruli and destroy it.

The immunoreactive group can be attached to the polymer by methods known in the art for derivatizing any functional groups typically found on proteins, or other immunoreactive groups. However, it is specifically contemplated that the immunoreactive group can be a nonprotein biomolecule. Thus, a linking group precursor useful in preparing the polymer of the invention will include those groups which can react with any biological molecule containing an immunoreactive group, whether Or not the biological molecule is a protein, to form a linking group between the polymer agent and the immunoreactive group.

Preferred linking group precursors can be selected

from but are not limited to:

(1) A group that will react directly with the amine or sulfhydryl groups on the protein or biological molecule containing the immunoreactive group, for example, active halogen containing groups including, for example, chloromethylphenyl groups and chloroacetyl [Cl-CH_2CO-] groups, activated 2-leaving group substituted ethylsulfonyl and ethylcarbonyl groups such as 2-chloroethylsulfonyl and 2-chloroethylcarbonyl; vinylsulfonyl; vinylcarbonyl; epoxy; isocyanato; isothiocyanato; aldehyde; aziridine; succinimidoxycarbonyl; activated acyl groups such as carboxylic acid halides; mixed anhydrides and the like; and other groups known to be useful in conventional photographic gelatin hardening agents.

(2) A group that can react readily with modified proteins or biological molecules containing the immunoreactive group, i.e., proteins or biological molecules containing the immunoreactive group modified to contain reactive groups such as those mentioned in (1) above, for example, by oxidation of the protein to an aldehyde or a carboxylic acid, in which case the "protein reactive group" can be selected from amino, alkylamino, arylamino, hydrazino, alkylhydrazino, arylhydrazino, carbazido, semicarbazido, thiocarbazido, thiosemicarbazido, sulfhydryl, sulfhydrylalkyl, sulfhydrylaryl, hydroxy, carboxy, carboxyalkyl and carboxyaryl. The alkyl portions of the protein reactive group can contain from 1 to about 18 carbon atoms. The aryl portions of the protein reactive group can contain from about 6 to about 20 carbon atoms.

(3) A group that can be linked to the protein or biological molecule containing the immunoreactive group, or to the modified protein as noted in (1) and (2) above by use of a crosslinking agent. Certain useful crosslinking agents, such as, for example, difunctional gelatin hardeners, bisepoxides and bisisocyanates become

a part of, i.e., a linking group in, the protein-segmented polymer complexing agent conjugate during the crosslinking reaction. Other useful crosslinking agents, however, facilitate the crosslinking, for example, as consumable catalysts, and are not present in the final conjugate. Examples of such crosslinking agents are carbodiimide and carbamoylonium crosslinking agents as disclosed in U.S. Patent 4,421,847 and the dication ethers of U.S. Patent 4,877,724. With these crosslinking agents, one of the reactants must have a carboxyl group and the other an amine, alcohol, or sulfhydryl group. The crosslinking agent first reacts selectively with the carboxyl group, then is split out during reaction of the "activated" carboxyl group with, for example, an amine to form an amide linkage between the protein or cytotoxic agent and the segmented polymeric chelating agent, this covalently bonding the two moieties. An advantage of this approach is that crosslinking of like molecules, e.g., proteins with proteins or complexing agents with complexing agents is substantially avoided where m is one, whereas the reaction of difunctional crosslinking agents is less selective and unwanted crosslinked molecules can be obtained. Especially preferred protein reactive groups include amino and-isothiocyanato.

While the segmented polymer according to the invention is described herein primarily in connection with its preferred utilities, i.e., as a contrast agent for use in imaging compositions and methods, it also finds utility in other applications and fields, e.g., as a therapeutic agent, an ion scavenging agent, an ion detecting agent and the like.

The segmented polymer according to the invention may comprise a therapeutic moiety and/or a moiety, preferably a metal, for enhancing contrast during imaging, and thus serve two functions simultaneously. This attribute is particularly useful in the detection

and treatment of tumors.

The segmented polymers according to the invention comprise a chelating agent residue linked to at least one poly(alkylene oxidylene) moiety. Higher molecular weight polymers comprising 100 to 750 alkylene oxide units are preferred in blood pool imaging as they have longer blood pool residence times.

The polymer may be prepared so as to be useful in imaging diseased tissue or state, i.e. useful in identifying or diagnosing the diseased area, and also useful in treating the area optionally in the same dose. This is accomplished by judicious choice of R which can be cytotoxic, and/or useful in imaging (e.g. fluorescence imaging if R=aryl) and of M, the chelated metal, which can be a cytotoxic radionuclide and/or an imaging agent useful in radioscintigraphy, x-ray, fluorescence ore imaging.

Viewed from a further aspect, the present invention provides a method of generating an enhanced image of the human or non-human animal body, said method comprising administering to said body a contrast enhancing amount of a contrast enhancing polymer according to the invention and generating an X-ray, MR, ultrasound or scintigraphic image of at least a part of said body into which said polymer distributes.

Viewed from a still further aspect, the present invention provides a method of therapy practised on the human or non-human animal body, which method comprises administering to said body a therapeutically effective polymer according to the invention.

Viewed from a yet further aspect, the present invention provides the use of the segmented polymers according to the invention for the manufacture of diagnostic or therapeutic agents for use in methods of image generation or therapy practised on the human or non-human animal body.

For magnetic resonance imaging applications, the

chelated metal ion $M^{(+a)}$ represents a paramagnetic metal ion such as for example an ion of atomic number 21 to 29, 42, 44 and 58 to 70. Ions of the following metals are preferred: Cr, V, Mn, Fe, Co, Ni, Cu, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm and Yb. Especially preferred are Cr^{3+}, Cr^{2+}, V^{2+}, Mn^{3+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Gd^{3+} and Dy^{3+}. Gd^{3+} and Dy^{3+} are most preferred.

For fluorescent imaging applications, M^{+a} represents a fluorescent metal ion, preferably a metal of atomic number 57 to 71, most preferably a Eu^{3+} ion.

$M^{(+a)}$ can be a radioactive metal ion isotope. As such, it can be a cytotoxic agent and/or an agent useful in diagnostic imaging such as in radioscintigraphy.

The radioactive metal isotope can be an ion of an isotope of a metal selected, for example, from the group consisting of Sc, Fe, Pb, Ga, Y, Bi, Mn, Cu, Cr, Zn, Ge, Mo, Tc, Ru, In, Sn, Re, Sr, Sm, Lu, Dy, Sb, W, Re, Po, Ta and Tl ions. Preferred isotopes of radioactive metal ions include ^{44}Sc, $^{64,67}Cu$, ^{111}In, ^{212}Pb, ^{68}Ga, ^{90}Y, ^{153}Sm, ^{212}Bi, ^{99m}Tc and $^{186,188}Re$ for therapeutic and diagnostic imaging applications.

Specifically contemplated are segmented polymers where r (the number of metals in the chelating residue) is greater than 1, and in those cases each $M^{(+a)}$ can represent the same or a different cation in the polymer; for example a polymer having $[Mn^{2+}]_2[Gd^{3+}]$ is specifically contemplated; as is $[Gd^{3+}]_2[^{67}Cu^{2+}]_2$ or any other combinations within or between the classes of cations used in the polymer. Such polymers are thus useful for more than one type of imaging or cytotoxic therapy. It is understood that the sum of positive charges ($\Sigma r.a$) is the sum of net positive charge for all cations. Thus for the listed examples above, $\Sigma(r.a)$ is 7 and 10, respectively. For example, if Z, the chelating agent residue, has 5 free carboxylates, (d = -5) and one Gd^{3+} chelated therein ($M^{(+a)} = Gd^{3+}$; a = +3), then the total charges of counter ion (w x b) must be +2, thus if w is

1, b is +2 as in calcium or magnesium; if w is 2, b is +1 as in a proton (assumed ionic for our purposes in formula I), sodium or potassium. If Z has d = -3 and chelates Gd^{3+} (a = +3), no counter ion is required, but they may exist if $\Sigma(w \times b) = 0$.

When E is present, i.e., when w is not zero, b most preferably is 1 or 2. E is especially preferably a pharmaceutically acceptable cation or anion. The skilled artisan will have no difficulty in selecting appropriate counterions. The total positive charge on the cations $\Sigma(r.a)$ equals the sum of the total charge on the chelating agent residue (d), plus the total charge on any counterions E present (b). When r=0, then w=0. When r=1, w can be 0, 1, 2, 3, 4 or 5. In the most preferred embodiment, r is 1 and w is 0, a=d+b.

E in formula I can be one or more counterions. For example, E can be one or more anions, such as a halide; sulfate; phosphate; nitrate; and acetate and the like. E can be one or more cations such as Na^+, K^+, meglumine, and the like. For <u>in vivo</u> applications, particularly for diagnostic imaging applications, nontoxic physiologically tolerable anions and cations are, of course, desirable.

Where r is 0 the segmented polymers according to the invention can be used in chelation therapy to treat heavy metal poisoning, e.g. lead poisoning and the like. For treatment of heavy metal poisoning, the polymers are administered alone without chelated ions, or in some applications of metal poisoning treatment, some other metal ions such as calcium are chelated by the polymer prior to administration. It is contemplated that such polymers as non chelated materials, as completely chelated or as mixtures of non chelated and chelated polymers can be used to treat poisoning from such metal ions as Sc, Ti, V, Cr, Mn, Fe, Eu, Er, Pb, Co, Ni, Cu, Ga, Sr, Y, Zr, U, Pu, Tc, Ru, In, Hf, W, Re, Os, Dy, Gd, Hf, La, Yb, Tc, As and the like.

In a mixture of chelated and non chelated polymers of this invention, wherein a metal is associated with the chelating agent residue in some of the segmented polymers of the mixture, the metal content in the mixture can vary from about 0.1 up to about 12% based on the total weight of the polymer in the mixture. The mixture of segmented polymers preferably contains the metal in an amount of from 1% to 5%, more preferably 1-4% by weight. Such mixtures can be formed by treating unchelated polymer with enough metal ion to chelate to from 1% to 15% or preferably from 1% to 10% by weight of the mixture.

The concept of drug targeting has gained importance in recent years, especially for anticancer drugs, inasmuch as toxic side effects of anticancer drugs to normal cells are a primary obstacle in cancer chemotherapy due to lack of selectivity to cancer cells. However, the desirability of targeting a certain tissue type finds application in other areas as well. Drug targeting can be accomplished by conjugation of the drug with, or encapsulation in, a specific transporter to the target. Immunoreactive groups or materials such as proteins, saccharides, lipids and synthetic polymers have been used for such transporters. Antibodies have been most widely used of these perhaps due to their target specificity and wide applicability.

It is known in the art that the size and charge of the polymer, as well as potential interactions with blood components, can influence the biodistribution of the polymer. Thus, judicious polymer synthesis can result in passive tissue targeting by the polymer.

For example, the hydrodynamic radius of albumin is approximately 37 Å, its molecular weight is 67 Kd, and its charge is known. It is known that the average half life for albumin circulation through tissue is approximately 24 hours, but this half life is longer in some tissues and shorter in others. It is also known

that the lymphatic system recirculates albumin back into the circulation system. Moreover, the concentration of albumin in certain tissues is appreciable and in other tissues albumin is nearly absent altogether. The skilled artisan can prepare a polymer of approximately for same size (or preferably the same hydrodynamic radius) and charge, and expect a similar half life and concentration in tissues.

The skilled artisan will recognize that inflammation of tissues will perturb the normal physiology of that tissue and thus perturb the half life and concentration of macromolecules (such as proteins or the polymer of the invention) in the inflamed tissue or inflamed tissue site. Thus the polymer finds utility in imaging and/or treating such inflamed tissues or inflamed tissue sites.

The skilled artisan will also appreciate that the absence of a lymphatic system in a tissue will perturb the concentration and increase the half life of macromolecules in a tissue because no convenient mechanism (e.g. the lymphatic system) is provided for the scavenging of such macromolecules. Such is the case in growing tumors. One can deliver the polymer as a cytotoxic agent, and/or an imaging agent to the growing tumor surface based on size of the polymer and on vasculature of the surrounding or targeted tissue. Thus, dosing with the appropriate molecular weight polymer will result in accumulation of such polymer in the growing surface of the tumor.

The composition according to the invention can be prepared in water-soluble or water dispersable forms depending upon the intended application more preferably in an injectable form for diagnostic imaging or as a composition intended to be administered intravenously. Such compositions preferably have a molecular weight of at least 4,500, more preferably 4,500 to 40,000. The preparation of water-soluble compositions of at least

4,500 in molecular weight can be accomplished by conventional means by one skilled in the art.

Actual dosage levels of the active ingredient in the compositions according to the present invention may be varied so as to obtain an amount of active ingredient that is effective to obtain a desired therapeutic or diagnostic response. The selected dosage level therefore depends on the desired therapeutic or diagnostic effect, on the route of administration, on the desired duration of treatment and other factors.

The total daily dose of the compounds of this invention administered to a host in single or divided dose may be in amounts, for example, of from about 1 picomole to about 10 millimoles of cytotoxic agent per kilogram of body weight. Dosage unit compositions may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the particular disease being treated.

The dosages of the contrast agent used according to the method of the present invention will vary according to the precise nature of the contrast agent used. Preferably however, the dosage should be kept as low as is consistent with achieving contrast enhanced imaging and volumes minimized for IV drip or bolus injection. In this way, the toxicity potential is minimized. For most magnetic resonance contrast agents the current appropriate dosage will generally range from 0.02 to 3 mmol paramagnetic metal/kg body weight, especially 0.05 to 1.5 mmol/kg, particularly 0.08 to 0.5, more especially 0.1 to 0.4 mmol/kg. However, it is anticipated that the amount of contrast agent needed

will decrease as detection sensitivity in imaging machines increases. It is well within the skill of the average practitioner in this field to determine the optimum dosage for any particular magnetic resonance contrast agent by relatively routine experimentation, for both in vivo or in vitro applications.

Contrast agents may be formulated with conventional pharmaceutical or veterinary aids, for example stabilizers, antioxidants, osmolality adjusting agents, buffers, pH adjusting agents, etc., and may be in a form suitable for injection or infusion directly or after dispersion in or dilution with a physiologically acceptable carrier medium, e.g., water for injection. Thus the contrast agents may be formulated in conventional administration forms such as powders, solutions, suspensions, dispersions, etc. However, solutions, suspensions and dispersions in physiologically acceptable carrier media will generally be preferred.

The contrast agents may be formulated for administration using physiologically acceptable carriers or excipients in a manner fully within the skill of the art. For example, the compounds, optionally with the addition of pharmaceutically acceptable excipients, may be suspended or dissolved in an aqueous medium, with the resulting solution or suspension then being sterilized.

Parenterally administrable forms, e.g., intravenous solutions, should of course be sterile and free from physiologically unacceptable agents, and should have low osmolality to minimize irritation or other adverse effects upon administration. Thus, the contrast medium should preferably be isotonic or slightly hypertonic. Suitable vehicles include aqueous vehicles customarily used for administering parenteral solutions such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection and other solutions such as

are described in Remington's Pharmaceutical Sciences, 15th ed., Easton: Mack Publishing Co., pp. 1405-1412 and 1461-1487 (1975) and The National Formulary XIV, 14th ed. Washington: American Pharmaceutical Association (1975). The solutions can contain preservatives, antimicrobial agents, buffers and antioxidants conventionally used for parenteral solutions, excipients and other additives which are compatible with the contrast agents and which will not interfere with the manufacture, storage or use of products.

The present invention includes one or more of the segmented polymers of this invention formulated into compositions together with one or more non-toxic physiologically acceptable carriers, adjuvants or vehicles which are collectively referred to herein as carriers, for parenteral injection, for oral administration in solid or liquid form, for rectal or topical administration, or the like.

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions and sterile powders and lyophilizates for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglycol, poly(ethylene glycol), glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

These compositions may also contain adjuvants such as preserving, wetting, emulsffying, cryoprotecting, and dispensing agents. Prevention of the action of microorganisms can be ensured by various antibacterial

and antifungal agents, for example, parabens, chlorobutanol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. In some embodiments, prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

The process according to the invention may typically be carried out by reacting a reactive poly(alkylene oxidyl) species, such as a poly(alkylene oxidyl)amine with a chelating agent containing reactive functionality (such as an anhydride, for example) in a non-reactive solvent to form an amide bond. The poly(alkylene oxide) can be capped or uncapped.

The preferred reaction conditions, e.g., temperature, pressure, solvent, etc., depend primarily on the particular reactants selected and can be readily determined by one skilled in the art.

Suitable reactive poly(alkylene oxidyl) species useful in the preparation and linking to R groups as described above include terminally functionalized poly(alkylene oxidyl) amines, poly(alkylene oxidyl) hydrazines, poly(alkylene oxidyl) isocyantes, poly(alkylene oxidyl) aldehydes, poly(alkylene oxidyl) carboxylic acids, poly(alkylene oxidyl) vinyl sulfonyl ethers, poly(alkylene oxidyl) phosphates, poly(alkylene oxidyl) N-alkylaminophosphoramidates, poly(alkylene oxidyl) epoxides, poly(alkylene oxidyl) alkoxides, poly(alkylene oxidyl) sulfonates, poly(alkylene oxidyl) halides, and the like. The above-described poly(alkylene oxidyl) species are linear and functional at both termini or at one terminus and end capped at the other terminus, for example with an ether group or a protecting group such as an acyl group or such as a trityl or dimethoxy trityl group or other protecting

groups typically used in peptide synthesis. These protecting groups may be removed by conventional means to permit reactions to produce amine or carboxyl. These species are prepared by simple chemical transformations which are well known to those skilled in the art to effect changes in functional groups in known compounds when preparing polymers, in this case the polymers of the invention. For example, acylation of hydroxy or amino-substituted species to prepare the corresponding esters or amides, respectively; simple aromatic and heterocyclic substitutions or displacements; cleavage of alkyl or benzyl ethers to produce the corresponding alcohols or phenols; and hydrolysis of esters or amides to produce the corresponding acids, alcohols or amines, preparation of anhydrides, acid halides, aldehydes, simple aromatic nitration and reduction to amine and conversion to isothrocyarrate with thiophosgene, and the like as desired can be carried out.

Such transformations will also provide suitable chelating agents, reactive functional groups or chelating agents (and precursors thereof) containing reactive functionality when applied to functional groups in chelating agents, including for example, polycarboxylic acids in anhydride form, sulfonyl chlorides, alkyl sulfates, vinyl sulfones, N-hydroxysuccinimide and other reactive esters, and the like. Sulfonyl chlorides, alkyl sulfates, vinyl sulfones and the like can be reacted with diamines such as ethylene diamine in excess to form multiple chelating agent cores as described above, or, if desired, single chelating agents linked for example by sulfonamidoethylene alkyleneamineethylene, sulfonatoethylene and the like groups respectively, to amino groups. Similarly, amino acids such as glycine or carboxy protected amino acids such as methylesters will react at the amine sites of the amino acids with the above functional groups to provide chelating agents that

contain carboxylic acid groups linked to the chelator in the analogous fashion. These carboxylic acid groups can then be activated for reaction with amine containing poly(alkylene oxide) groups to form amide bonds. The amine containing chelators can react with carboxylic acid containing poly(alkylene oxide) groups or with active esters or anhydrides and the like thereof to form amide groups. Preferably there will be at least m reactive functional groups on the chelator so that m poly(alkylene oxidyl) amide bonds can be formed. Variations in the functional group type, for example, using a vinyl sulfone and then an activated hydroxysuccinimide or an anhydride plus a more reactive acyl-N-methyl imidazolium salt will permit sequential substitution of the chelator moiety by poly(alkylene oxidyl) amines having different molecular weights from each other. Alternatively, a chelator containing multiple functional groups such as anhydrides can be treated in sequence with less than stoichiometric amounts of different amine containing poly(alkylene oxidyl) moieties to provide amide link segmented polymers.

As will be recognized by one skilled in the art, obtaining the desired product by some reactions will be better facilitated by blocking or rendering certain functional groups inert. This practice is well recognized in the art, see for example, Theodora Greene, Protective Groups in Organic Synthesis (1991). Thus when reaction conditions are such that they may cause undesired reactions with other parts of the molecule, for example, in portions of the chelator intended to become ligands, the skilled artisan will appreciate the need to protect these reactive regions of the molecule and will act accordingly. The chelating agent residue containing reactive functionality can be prevented from from forming undesired products by suitably blocking the chelating agent residue precursor which can be contacted

with the reactive poly(alkylene oxide) moiety to form the polymer, and then the blocking group can be subsequently removed by techniques known in the art. For example, if hydroxy substituents are to be selectively present in the final polymer, they preferably should be temporarily blocked during the segmented formation polymer, such as formation of an alkyl or trityl ether from the hydroxyl by conventional blocking techniques to minimize formation of undesirable by products and then deblocking with, for example BBr_3 or CF_3COOH, respectively, after formation of the segmented polymer. However, by products which contain one or more linkages formed by unblocked reactive precursor groups in the backbone of the polymer are contemplated to be useful.

Suitable amine functionalized reactive poly(alkylene oxidyl) species can be prepared by methods known in the art. For example, a preferred poly(alkylene oxidyl)amine can be prepared by reacting an activated form of the poly(alkylene oxide) such as halide or sulfonate ester with ammonia, a primary amine, an amide or an azide followed by reduction. Alternatively, the amino group can be introduced by other methods known in the art (see Leonard et al, Tetrahedron 40, 1581-1584 (1984); David et al., U.S. Patent No. 4,179,337; Gerhandt et al., Polym.Bull, 18, 487-93 (1987)). Suitable illustrative amines include N-methylamine, amino acids such as glycine and the like, aminomethyl pyridine, aminomethylthiophene, methoxyethoxyethylamine, methoxyethylamine and aminobenzoic acid. Poly(alkylene oxidyl) amines are commercially available, known in the art, or can be prepared by known methods, including those described herein.

The activated form of the poly(alkylene oxide) is reacted preferably with a stoichiometric excess of an amine, in an inert solvent preferably at a temperature (e.g., 100-160°C) and pressure (e.g., 1 to 10 atmospheres) sufficient to drive the reaction to

completion. Suitable inert solvents include dioxane, DMF, ethanol, or other alcohols and the like. Thereafter, the poly(alkylene oxidyl)amine preferably is isolated, e.g., by evaporation or precipitation, and purified, e.g., by dissolving in a suitable solvent such as methylene chloride, chloroform or trichloroethane, and then washed with an excess of aqueous NaOH, or by any other suitable isolation and purification techniques available to the skilled artisan.

Suitable carboxyl functionalized reactive poly(alkylene oxidyl) species can be prepared by methods known in the art. For example, a preferred poly(alkylene oxidyl) carboxylic can be prepared by reacting a hydroxyl containing poly(alkylene oxide) with a cyclic anhydride or with chloro- or bromo- or iodoalkyl acids such as chloroacetic acid and the like using potassium carbonate as a base. The terminal hydroxy group can also be oxidized to a carboxylic acid group.

Alternatively, the amine containing poly(alkylene oxides) above can be elaborated by similar chemistry into carboxylic acid containing poly(alkylene oxides). Additionally, reaction of a haloalkyl poly(alkylene oxide) or with a poly(alkylene oxidyl) sulfonate ester with an amino acid as above provides a carboxylic acid containing poly(alkylene oxide). These materials can be purified and isolated using techniques outlined above. These carboxylic acid containing poly(alkylene oxides) or activated derivatives thereof can react with amine containing chelating agents to form amide groups in the preparation of the segmented polymers of this invention.

Materials for preparing the prefered embodiment, such as the internal anhydride forms of the chelating agents described above, are commercially available and/or can be prepared by techniques known in the art. For example, the internal anhydride form OTPA is commercially available. The internal anhydride forms of B4A, P4A and TMT can be prepared by techniques known in

the art. For example, the anhydrides can be prepared by heating the corresponding acids in acetic anhydride in the presence of pyridine as catalyst. Mixed anhydrides of chelators are also suitable for segmented polymer formation.

In a preferred embodiment the reactive poly(alkylene oxidyl) amine can be reacted with the internal dianhydride in a suitable solvent to form the unmetallized composition. The reaction conveniently can take place at approximately room temperature and atmospheric pressure. However, higher and lower temperatures and pressures are contemplated. Suitable solvents include dimethylsulfoxide, dimethylformamide, acetonitrile, chloroform, dichloromethane, 1,2-dichloroethane and other aprotic solvents. The nonmetallized polymer preferably is isolated and then purified, e.g., by diafiltration or other methods available to the skilled artisan. Reaction without solvent in molten polyalkylene oxide is contemplated.

Alternatively, the segmented poiymer can be prepared in a condensation polymerization reaction between a suitable poly(alkylene oxidyl) amine and a metallized chelating group containing carboxylic acid functionality not participating in the chelators, in a suitably activated form.

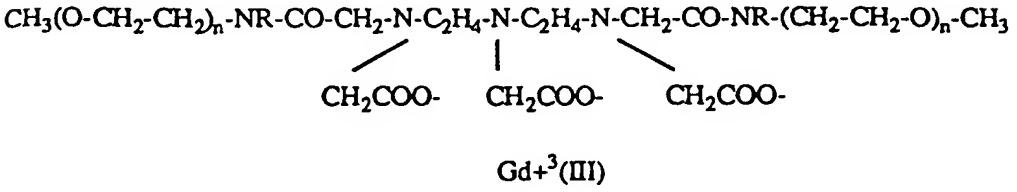
In the preferred embodiment, the metallized polymer can be formed by contacting the unmetallized polymer sequentially or simultaneously with one or more sources of metal ions. This can be conveniently accomplished by adding one or more metal ion solutions or one or more metal ion solid salts or metal ion oxides, preferably sequentially, to a solution, preferably an aqueous solution, of the polymer. Thereafter, or between sequential addition of metal ions, the chelated polymer preferably is diafiltered in water to remove excess unbound metal or can be isolated by other methods known in the art.

Viewed from a still further aspect, the present invention provides a process for the preparation of a chelated metal bearing segmented polymer, said process comprising metallating a chelating agent residue containing polymer according to the invention eg by admixing the polymer in a solvent together with an at least sparingly soluble compound of the metal, for example a chloride, oxide, acetate or carbonate.

Examples

The following examples illustrate the invention in a non-limiting fashion.

Examples were prepared by the reaction of Ω-methoxy-α-amino poly(ethylene oxide) (PEO-NH_2) and Ω-methoxy-α-methylamino-poly(ethylene oxides) (PEO-$NHCH_3$), having molecular weights from 2000 to 10,000 with diethylenetriamine pentaacetic acid (DTPA) dianhydride in chloroform in the presence of a catalyst, 1,8-diazabicyclo-(5,4,0)-undec-7-ene. Complexation of the Gd^{3+} ion was accomplished by adding excess $GdCl_3$ to the polymeric chelate composition in aqueous solution. Amount of complexation was determined by potentiometric titration of acid groups and by neutron activation or ICP-AES of Gd(157). Examples have the structure;



R=H, CH_3

Example 1

5g of PEO-NH_2 (Sigma Chemical Co., St. Louis, MO) (5,000) (1mmol) was mixed with 0.178g of DTPA dianhydride (Aldrich Chemical Co., Milwaukee, WI) (0.5 mmol) and 5 drops of the catalyst in 200 mL of chloroform. The mixture was refluxed at 60°C for three days. The solvent from the reaction solution was removed by rotatory evaporator. The reaction product was then dissolved in water and acidified to pH 2.5 with concentrated HCl to convert any unreacted DTPA dianhydride to acid. The solution was exhaustively dialyzed in a 6000-8000 MW

cut-off dialysis bag, followed by diafiltration using an Amicon® ultra filtration unit with 10K cut off filter, to remove any unreacted DTPA and PEO-NH_2. The DTPA-conjugated polymer was recovered by freeze drying, yielding 4.8 g solid. A sample of the solid polymer (MW~11,000) was used to determine the number of acid groups per polymer chain. A pH titration was performed with the pre-complexed polymer to determine the composition of the polymeric chelate. The number of acid groups per polymer chain (MW~11,000) was determined to be three, indicating that two PEO moieties were attached.

A small amount of the polymer was dissolved in water and mixed with three fold molar excess of $GdCl_3$. The solution was adjusted to pH 5.0 with NaOH and subjected to diafitration to remove excess $GdCl_3$, and the solid was recovered by freeze drying. The amount of Gd in the final complex was determined by neutron activation.

The neutron activation results in 0.01236g Gd/g polymer compared to calculated value of 0.01427g Gd/g polymer.

Example 2

(A) 3.93 grams (0.01 mol.) of DTPA (pentaacetic acid form) was suspended in 4.85 mL of pyridine and 3.57 grams of acetic anhydride was added. The mixture was heated to 65°C. After cooling, the precipitate was filtered off and vacuum dried at 40°C, yielding 2.11 g. (87%) which was used unpurified in the next step.

(B) Ω-methoxyPEO tosylate (2000 MW) was suspended in 20 mL dioxane in a reaction bomb, cooled in an ice bath and methylamine bubbled into the mixture over 15 minutes. The bomb was sealed and heated to 160°C in an oil bath for 18 hours. Upon cooling the solution was filtered and concentrated; the residual solid was dissolved in 40 mL

of water +2.0 mls of 1N NaOH. The solution sat at room temperature for 30 minutes, and was extracted twice with chloroform; the extracts were dried over magnesium sulfate and concentrated in vacuo to a tan solid. Yield 1.54 g.

(C) The DTPA anhydride from example 2A and the Ω-methoxyPEO methylamine from example 2B are combined in 60 mL of chloroform and treated with six drops of 1,8-diazbicyclo[5.4.0]undec-7-ene DBU and heated in an oil bath at 60°C for three days. The resulting product was concentrated in vacuo to yield 1.92 g. of a solid. The solid produced was dissolved in 55 mL of water, and diafiltered in a 200 mL Amicon diafiltration cell with a 5000MW cut off membrane. Approximately 700 mL of diafiltrate was collected. The retentate was filtered through a 0.2 micron filter and concentrated in vacuo at 1.5 torr and freeze dried to yield 1.183 g polymer solid.

(D) The product in example 2C was dissolved in 100 mL of water containing 0.698 g. of $GdCl_3.6H2O$. The yellow solution was stirred at room temperature for 1 hour and then was placed in a 200 ml. Amicon diafiltration cell with a 5000MW cut-off membrane and diafiltered with water. 725 ml of diafiltrate was collected, and the yellow retentate was filtered through a 0.2 micron filter and freeze dried; yield 3.48 g; 4.38% Gd by ICP analysis.

Example 3

(A) Ten grams of Methoxy(PEO-OH(5000MW) in 100 mL pyridine was combined with a mixture of 4.0 g. of tosyl chloride and 4.1 g of 2,6-di-t-butyl-4-methylpyridine in 10 mls of pyridine. The resulting solution was stirred at room temperature under nitrogen for 5 hours, then poured into a mixture of 100 mls of concentrated HCl and

ice, extracted twice with chloroform and dried over magnesium sulfate and concentrated in vacuo to a white solid which was triturated overnight in ether. The solid was filtered, washed with ether, and vacuum dried to 9.79 g (95%) of the desired product. The resulting tosylate was then aminated as in Example 2B with methyl amine.

(B) 0.293 g.of DTPA anhydride was combined with Ω-methoxy methylamino PEO (9.79 g.) in 390 mL of 1,2 dichloroethane and 10 drops of DBU was added dropwise the mixture was heated in oil bath at 60°C under nitrogen for three days. The reaction mixture was cooled to room temperature, concentrated in vacuo to 10.42 g of a tan yellow solid which was taken up in 180 mL of water, filtered, and placed in a 200 mL Amicon diafiltration cell with a 10,000 molecular weight cut-off membrane, and diafiltered with water at room temperature. Diafiltration was stopped after 1150 mL of diafiltrate was collected, and the retentate was freeze dried to yield 5.39 g of an off-white fluffy solid of approximately 10,000 molecular weight.

(C) 2.00 g of the product of example 3b was dissolved in 50 mL of water and 0.132 g $GdCl_3$ hexahydrate was added. The solution was stirred at room temperature for one hour followed by diafiltration in a 200 mL Amicon diaflltration cell (5000 MW cut-off membrane) versus water. After collecting 700 mL of diafiltrate, the retentate was filtered and freeze dried to yield 1.81 g of product as a fluffy white solid of approximately 10,000 mol. weight; 1.18% Gd by ICP analysis.

Example 4

MethoxyPEO-OH (molecular weight 10,000) was aminated using the method of example 2B and then reacted with DTPA anhydride according to the method of example 2C.

This material (4.12 g) was dissolved in 100 mL of water containing 0.149 g of $GdCl_3$ hexahydrate and stirred at room temperature for one hour, followed by diafiltration, yielding 4.106 g of a fluffy white solid; 0. 763% Gd by ICP analysis.

Example 5

(A) It is contemplated that a chelating residue comprising two DTPA residues is prepared by reacting the free carboxylic acid of DTPA anhydride with carbonyl N,N^1- dimethyldrimidazolium ditriflate at reduced temperature in an inert atmosphere followed by treatment with ethylene diamine to provide a tietraanhydride diamide.

(B) The resulting anhydride from 5A is then reacted with methoxy PEGamine according to the method of Examples 1-3 to form a polymer of Formula I.

Example 6

(A) It is contemplated that a chelating agent comprising three DTPA residues is prepared by reacting DTPA dianhydride with carbonyl N,N^1-dimethyldrimidazolium ditriflate as above followed by treatment with diethylene triamine to provide a hexaanhydride triamide.

(B) The resulting anhydride from 6A is then reacted with methoxy PEG amine according to the method of Examples 1 to 3 to form a polymer of formula I.

Each of the examples 1 through 4 were tested for blood pool retention time in rodents, against MAGNEVIST, $DTPA/Gd^{3+}$, a known imaging compound. Retention time in the chart below is reported as the blood pool half life,

T 1/2 (minutes) for half of the composition to be depleted from the blood pool.

	MW of Composition	Blood Pool T1/2 (Minutes)
(Magnevist®)	0.9 K	12
2	4.5 K	15.6
3	11 K	15.3
4	21 K	82.9

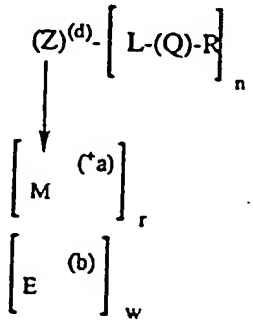
CLAIMS

1. A segmented polymer having a molecular weight of at least about 4,500 comprising at least one poly(alkylene oxidylene) group linked via an amide linkage to a chelating agent residue.

2. A polymer as claimed in claim 1 wherein said segmented polymer is metallated.

3. A polymer as claimed in either of claims 1 or 2 wherein said segmented polymer is metallated with a paramagnetic metal species or with a metal radionuclide species.

4. A polymer as claimed in any one of claims 1 to 4 having the formula:



(wherein:

Z is a chelating agent residue;

Q is a divalent poly(alkylene oxidylene) moiety having a carbon terminus at R and at L;

L represents an amide linkage;

$E^{(b)}$ is one or more counterions each having a charge of b;

b is an integer from 1, 2 and 3;

n is an integer from 1, 2, 3 and 4;

w is zero or an integer from 1, 2, 3, 4 and 5;

$M^{(+a)}$ is a cation, having a charge of +a;

a is an integer from 1, 2, 3 and 4;

r is 0 or an integer from 1, 2 and 3, with the proviso that when r is 2 or 3, each $M^{(+a)}$ can be the same or different cation;

d is the total charge on the chelating agent residue and is an integer from 0 to 10;

$d + \Sigma(b.w) + \Sigma(a.r)=0$; and

R is a capping moiety selected from the group consisting of hydrogen, hydroxyl, C_{1-4}-alkyl, C_{6-24}-aryl carbon atoms, C_{2-5}-alkanoyloxyl and C_{1-4}-alkoxy, or R is an immunoreactive group or cytotoxic drug linked to Q by a chemical bond or a linking group).

5. A polymer as claimed in any one of claims 1 to 4 having a molecular weight of between 10,000 and 40,000.

6. A polymer as claimed in any one of claims 4 to 5 wherein r is 1, 2 or 3 and each cation is a paramagnetic ion.

7. A polymer as claimed in any one of claims 4 to 6 wherein Z comprises one or more units of DTPA.

8. A polymer as claimed in any one of claims 4 to 7 wherein Q is a poly(ethylene oxidylene) moiety.

9. A polymer as claimed in any one of claims 4 to 8 wherein $M^{(+a)}$ is selected from the group consisting of Gd^{3+}, Fe^{3+}, Mn^{2+}, Mn^{3+}, Dy^{3+} and Cr^{3+}.

10. A polymer as claimed in any one of claims 4 to 9 wherein at least one cation (M^{+a}) is a metal radionuclide ion.

11. A polymer as claimed in claim 4 wherein Z is a residue of DTPA, $M^{(+a)}$ is Gd^{3+} or Dy^{3+}, Q is a poly(ethylene oxidylene) moiety of MW 2000-20,000, R is a hydroxyl or methoxy group, and n is 2.

12. A polymer as claimed in claim 4 wherein R is an immunoreactive group, r is at least 1, and $[M^{+a}]$ is a cation selected from the group consisting of a radionuclide ion, a fluorescent metal ion, and a paramagnetic ion.

13. A polymer as claimed in claim 12 wherein the immunoreactive group is selected from the group consisting of a histone; DNA, including antisense DNA; RNA, including antisense RNA; actin or myosin; or an antibody to DNA, RNA, histones, actin or myosin.

14. A pharmaceutical composition comprising a segmented polymer as claimed in any one of claims 1 to 13 together with at least one physiologically acceptable carrier or excipient.

15. A process for preparing a segmented polymer as claimed in any one of claims 1 to 13, said process comprising reacting a poly(alkylene oxide) with a chelating agent or precursor thereof.

16. A method of generating an enhanced image of the human or non-human animal body comprising administering

to said body a contrast enhancing amount of a contrast enhancing polymer as claimed in any one of claims 1 to 13 optionally together with a pharmaceutical carrier or excipient and generating an image of at least a portion of said body into which said polymer distributes.

17. Use of a polymer as claimed in any one of claims 1 to 13 for the manufacture of a diagnostic or therapeutic agent for use in a method of image generation or therapy practised on the human or non-human animal body.

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/GB 95/00742

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K49/00 A61K47/48 C08G65/32

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,94 08624 (STERLING WINTHROP INC.) 28 April 1994 *Whole document* ---	1-17
P,X	WO,A,94 09056 (STERLING WIMTHROP INC.) 28 April 1994 *Whole document * ---	1-17
P,X	WO,A,94 08629 (STERLING WINTHROP) 28 April 1994 *Whole document* ---	1-17
X	WO,A,93 06148 (UNGER E. ET AL) 1 April 1993 see claims 1,2,5,10,21,22 see page 14, line 5 - line 15 see page 13, line 17 - line 35 --- -/--	1-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 1995	03.07.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	O'Sullivan, T

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INTERNATIONAL SEARCH REPORT

Interna. al Application No
PCT/GB 95/00742

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	EP,A,0 326 226 (NYCOMED AS) 2 August 1989 see claim 1 ---	1
A	US,A,5 283 339 (ARNOLD F.A.) 1 February 1994 see claim 1 -----	1

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Interna ..al Application No
PCT/GB 95/00742

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US-A-5283339	01-02-94	NONE		